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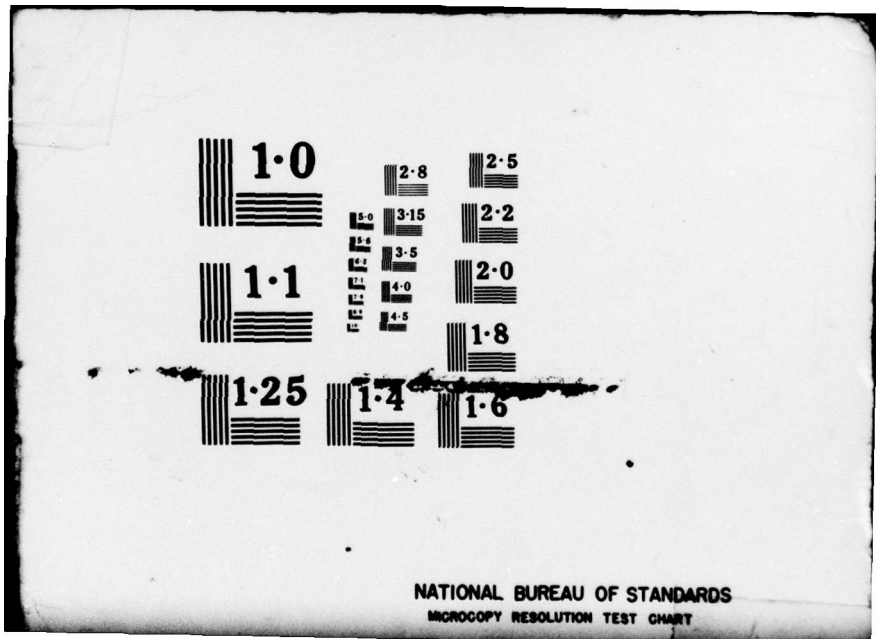
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Annual Progress Report No. 5

Genetics of Novel Hybrid Bacteriophage and Development of Generalized Transducing System for Salmonella typhosa

Annual Progress Report
(From 1/1/78 to 8/31/78)

Nobuto Yamamoto, Ph.D.

August, 1978

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND,

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Various hybrid phages between Salmonella Phage P22 and coliphages such as λ and φ80 have been isolated by using their common bacterial hosts, E. coli-S. typhimurium hybrids. Among those hybrid phages, λ-P22dis and φ80-P22 hybrid classes carry a large genetic segment of P22 phage containing the Im

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20. Abstract (continued)

gene in addition to the c genes. Since the Salmonella somatic antigen conversion gene al and tail component gene 9 of P22 are located between the c and Im genes, these hybrid phages should carry the al and tail gene 9 of P22. Therefore we studied the antigen conversion of various bacterial hosts and the P22 tail gene expression, although these genes are dispensable for these hybrid phages.

Both λ-P22dis and ϕ80-P22 can convert E. coli-S. typhimurium with somatic antigen O-1. However, E. coli K12 was not converted by these hybrid phage classes. This can be explained by the fact that E. coli does not have O-1 antigen acceptor sites which are [gal-rha-man] repeating units of Salmonella typhimurium cell surface. Moreover, the P22 tail component gene 9 in λ-P22dis is expressed during lytic replication of λ-P22dis hybrid phage.

Since the att region of P22 is located between the c and Im genes, we analyzed the prophage insertion site of λ-P22dis using E. coli K12 derivatives with a pro AB deletion and a plasmid carrying F lac+ pro+ att P22+. The λ-P22dis phage was found to carry the att region of P22.

From these observations, it is evident that λ-P22dis carries a large P22 genetic segment containing the Im through h21 genes. The physical map of λ-P22 dis genome was studied by electron microscopic DNA heteroduplex method. The P22 segment is situated at the central region of the λ-P22dis genome and splits λ segments. About 40% of the λ-P22dis genome was derived from P22 genome while the remaining 60% from λ genome. This physical map was also substantiated by EcoR1 restriction endonuclease analysis.

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Summary

We mapped the phage chromosomes of hybrids between Salmonella phage P22 and coliphages. Since the genomes of hybrid phages consist of clusters of genes derived from evolutionary diverse bacteriophages, we studied the physical length of the homology between parental phages and hybrid phages and controls of various gene expressions in these hybrid phages. In this progress report we showed the origin of genetic segments in the hybrid phage genomes and the expression of dispensable genes such as the somatic antigen conversion gene al and the P22 tail component gene 9 in these hybrid phages.

Foreword

Fundamental studies of viral genetics not only play an important role in increasing our knowledge of the action of viruses in disease processes, but have contributed greatly to our knowledge of the whole problem of cell replication, genetic transfer, gene control, morphogenesis, and antigen conversion. The significance of the study of bacterial hybrids between E. coli and Salmonella has greatly broadened with the recent discoveries of hybrid phage between coliphage and Salmonella phage. The study supported by this contract will bring many important answers for mechanisms of genetic evolution, transduction, recombination, gene expression, antigen conversion and viral replication. In addition, such newly constructed hybrids may prove useful in achieving intergeneric transduction via a hybrid phage vector, of chromosomal genes from different genera of enterobacteriace. Therefore such hybrid phages may serve as useful vectors in the genetic engineering of a polyvalent oral attenuated vaccine which expresses immunogenic determinants for antigens of Shigella, Salmonella and perhaps even cholera.

Progress

Present Status of this Project

We have previously reported the isolation of an unusual Salmonella typhimurium hybrid sensitive to coliphage λ and Salmonella phage P22 (Gemski, Baron and Yamamoto, PNAS 69, 3110, 1972). This hybrid, constructed by mating an Escherichia coli K-12 Hfr donor with an S. typhimurium recipient, was characterized as an excellent host for achieving genetic recombination between λ and P22. Two broad hybrid phage classes, each with representative types differing presumably in the extent of gene exchange, have been isolated and described in our previous reports. The λ-P22 hybrid class, which has the protein coat of λ, was found to contain at least the c region of P22. The other class, termed P22-λ, has the protein coat of phage P22, and has inherited at least the c marker of λ.

By employing an approach similar to that previously used to map homologous chromosomal regions of P22 and P221 (Virology 28, 168, 1966), we have studied representatives of the λ-P22 class and determined the extent of their genetic recombination. λ-P22 type 1 hybrids have replaced the int through Q chromosomal segment of λ with functionally related P22 genes, this region representing approximately 25% of the λ genome. In λ-P22 type 2 hybrids, however, a shorter segment containing int through P of λ have been replaced by P22 genes. Similarly, we have studied representatives of the P22-λ class and determined the extent of their genetic recombination. Representatives of the P22-λ phage class, selected for inheritance of the c region of λ during recombination between genetically marked λ and P22 derivatives have been characterized by genetic procedures. P22-λ type 1 hybrids have replaced the c through gene 12 chromosomal segment of P22 with functionally related λ genes carrying the c through P genes. P22-λ type 2 hybrid, however, have replaced the c gene segment of P22 with the corresponding

λ genes containing the cI, cII, cIII and N genes.

We have also isolated hybrids between Salmonella phage P22 and coliphage φ80. These hybrid phages provide excellent models for studying a mechanism of genetic evolution, control of gene expression within gene clusters derived from diverse phages, phage morphogenesis, chromosome structure and nature of transduction. The hybrid phages may be used for intergeneric transduction of chromosomal genes from different genera of the enterobacteriace. Consequently, a new system for investigating, for a genetic point of view, the pathogenesis of distinct enteric infections (for example, salmonellosis vs colibacillosis) is now feasible. Such hybrid phages, besides being transductional vectors of chromosomal genes, could also achieve antigenic conversion of various Salmonella determinants on an intergeneric level.

1. An unusual hybrid phage between coliphage λ and Salmonella phage P22

As we reported previously, the genetic homology between P22 and λ-P22 has been analyzed by recombination experiments. The length of the homologous region between P22 and λ-P22 varied among many independent λ-P22 isolates. Although P22 and λ-P22 share c regions, most λ-P22 lysogens are not immune to P22 infection. However, one of λ-P22 groups, λ-P22dis, conferred the hosts immunity to superinfection with P22 and formed plaques on bacterial strains lysogenic for λ-P22. Therefore it is evident that P22 supplies an additional marker Im (immunity), as well as the c, g and h2l markers to form λ-P22dis. It should be mentioned here that λ-P22 lysogens are not only immune to λ-P22 but also immune to another hybrid phage species P22l, carrying the c region of P22. Although the c region of P22 is enough to confer immunity to these phages, the c region alone is not enough to establish immunity to P22 and λ-P22dis infection. Thus the Im region of P22 is required for establishing the immunity to P22 and λ-P22dis superinfection. The finding of various λ-P22 types provides a crucial demonstration of the second region responsible for establishing the immunity to phage P22.

The above finding implies that the length of homology between P22 and λ-P22dis group is longer than that between P22 and λ-P22. Thus, it is unequivocally concluded that P22 supplies various lengths of its genetic segment to form a variety of λ-P22 types. From these observations, it should be concluded that λ-P22 arises as a consequence of recombination between P22 and λ though no evidence for genetic homology between these phages was observed. Thus, the genome of λ-P22 consists of parts of P22 and λ. The length of P22 segment inserted into λ-P22 groups varies from strain to strain of λ-P22 groups.

2. Physical Lengths of Homologous and Nonhomologous Regions between λ and λ-P22dis: Electron Microscopic Heteroduplex Method.

The DNA of hybrid phage λ-P22dis was examined in heteroduplex studies with λ DNA to provide confirmation of our genetic findings on the extent of phage recombination. A number of heteroduplex molecules formed by hybridization of single strands of DNA from λ and λ-P22dis were observed and measured in electron micrographs. Large continuous regions of homology are evident at both ends of the DNA molecule, suggesting that the λ-P22dis has a unique genome structure and the fixed ends of λ phage. In contrast, the center of the heteroduplex was found to contain large regions of non-homology interrupted by short double stranded regions. The regions of non-homology amount to about 40% of the total length of the molecule indicating that the 40% segment is derived from P22.

3. Restriction Endonuclease Analysis of DNA from λ-P22 Hybrid Phages

The λ-P22 hybrid phages in which segments of the P22 genome have replaced portions of the λ genome were examined by restriction endonuclease analyses. The extent of substitution of P22 into the λ genome was also determined by electron microscope heteroduplex studies. All of the hybrids examined have the P22 segment substituted into the central region of the λ genome. Fragments that result from EcoRl restriction enzyme digestion of the DNA from these λ-P22 hybrids were compared to the restriction fragments obtained from λ and P22 DNA. Most of the fragments from the hybrids are identical to those obtained from λ, but at least one or more correspond to P22 fragments. Thus, the substitution of a P22 segment into λ results in the loss of the EcoRl fragments of λ and the appearance of P22 fragments. A correlation can, therefore be made between map position of λ fragments and the corresponding

P22 fragments that replace them. One of the hybrids, λ-P22*dis*, contains a large segment of substituted P22 DNA and restriction analysis was used to order the P22 fragments in this segment.

4. *Lysogenization and Prophage Attachment Site of λ-P22dis.*

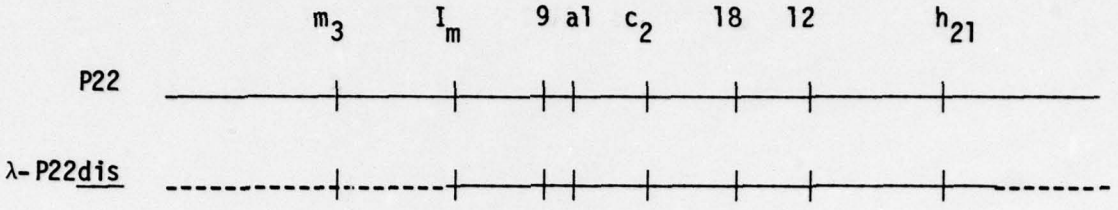
The homology between λ-P22*dis* and P22 extends from *h21* to *Im* genes. This large homology suggested that phage λ-P22*dis* might be integrated at the (preferred) prophage integration site for P22, near *pro* A.

An *E. coli* K12 mutant in which a segment including the *pro* AB region is deleted, CGSC4288*mal*$^+$ could not be lysogenized by λ-P22*dis*. From these results, it may be suggested that P22 and λ-P22*dis* phages share the prophage attachment site of the host bacterial chromosome.

In order to confirm this conclusion, a derivative of *E. coli* K12#CGSC4288*mal*$^+$ which carries a plasmid F *lac*$^+$ *pro*$^+$ *attP22* was therefore tested for susceptibility to lysogenization by λ-P22*dis*. This plasmid carrier was readily lysogenized by both of these phages.

Six λ-P22*dis* lysogenic derivatives of the plasmid carrier were isolated with six independent λ-P22*dis* strains. They were then cultured in nutrient broth for 20 hrs, and plated on MacConkey agar containing 0.1% lactose. After overnight incubation at 37°C, the total of 19 *lac*$^-$ segregants, average of three segregants from each of these lysogens were isolated and tested for lysogenicity and *pro*$^-$ phenotype. All nineteen segregants were found to be *pro*$^-$ and not lysogenic for λ-P22*dis*, suggesting that those lost the plasmid also lost λ-P22*dis* prophage. Therefore it is unequivocally concluded that λ-P22*dis* carries the *attP22* gene of P22 and thus sharesthe prophage attachment site of P22 near *pro* A gene of host bacterial chromosome.

Figure 1. Genetic structure of P22 and λ-P22dis



5. Antigen conversion gene of λ-P22dis hybrids

λ-P22dis hybrids form plaques on λ-P22 lysogens, because the left end of the P22 segment in the λ-P22dis extends to the left of the Im gene of P22. As shown in Figure 1, the antigen conversion gene al of P22 is located between c3 and Im genes of P22. Accordingly, we tested the antigen conversion of various bacterial strains lysogenic for λ-P22dis and found that about 100% of λ-P22dis strains conferred antigen 01 conversion to E. coli-S. typhimurium hybrid WR4027.

6. Antigen conversion of E. coli by a λ-P22 hybrid phage strain.

As we reported previously, we have isolated phage λ-P22dis, an unusual hybrid between P22 and λ. This hybrid carried a large P22 genetic segment containing Im (the 2nd immunity gene) as well as c, g and h21 genes. In addition we found that some λ-P22dis strains also carry the antigen O-1 conversion gene al of P22. Therefore they can confer antigen O-1 conversion to E. coli-S. typhimurium strain WR4027. However they do not convert E. coli K12. This can be explained by the fact that E. coli K12 does not have O-1 antigen acceptor sites which are [gal-rha-man] repeating units of Salmonella typhimurium cell surface. When E. coli K12 derivatives carrying a small Salmonella genetic segment for the [gal-rha-man] repeating units were examined, they were readily converted by the λ-P22dis strains.

7. Antigen conversion of E. coli-S. typhimurium hybrids by ϕ80-P22 hybrids.

Phage ϕ80-P22 is a hybrid type between coliphage ϕ80 and Salmonella phage P22. All ϕ80-P22 strains isolated carry the antigen conversion gene as well as Im, c and h2l genes of P22. Therefore these ϕ80-P22 strains are able to convert E. coli-S. typhimurium strain WR4027 and E. coli K12 carrying the repeating units of Salmonella typhimurium.

8. Expression of the P22 Tail Gene 9 in λ-P22dis hybrids.

Since λ-P22dis carries the P22 tail gene 9, it was desirable to see whether the gene 9 is expressed during λ-P22dis replication. In order to test expression of the gene 9, the in vitro self-assembly method of Israel, Anderson and Levine (Proc. Nat. Acad. Sci. 57, 284-291, 1967) was used.

When S. typhimurium Q (2×10^8 cells/ml) was infected with a temperature sensitive mutant of P22 gene 9 and cultured for 1 hour at 39°C, according to Israel, Anderson and Levine the resulting lysate theoretically should contain about 2×10^{10} tail-less P22 head particles which still contain intact whole P22 genome. When this head donor preparation was added to the lysates of λ-P22dis previously grown in E. coli-S. typhimurium hybrid WR4027 and incubated for 1 hour and plated on a P22 specific indicator strain Q or Q/22l at 25° because P22 head preparation carried a genome with temperature sensitive tail gene, about a 10,000 fold increase of P22 plaque forming activity was found. This increased plaque formation was completely inhibited by using λ-P22dis lysate pretreated with anti-P22 but not affected by λ-P22dis lysate pretreated with anti-λ. From these observations it is concluded that the gene 9 of P22 in λ-P22dis was expressed.

Publications

Yamamoto, N., Wohlhieter, J.A., Gemski, P. and Baron, L.S. λimmP22dis: A hybrid of coliphage λ with both immunity region of Salmonella phage P22, Abt. Am. Soc. Microbiol., p. 259, 1978.

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